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A MULTIPLEXED IMMUNOASSAY FOR DETECTION OF ANTIBODIES TO ACTINOBACILLUS PLEUROPNEUMONIAE IN PIGS

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INTRODUCTION:

The bacterium Actinobacillus pleuropneumoniae (App) is the causative agent of porcine pleuropneumoniae, a contagious and severe respiratory disease in pigs. Based on capsular antigens, 15 App serovars have been described, and the prevalence and morbidity of these serovars vary with geographic regions (1). In Denmark, the most important serovars are considered to be App 1, 2, 5, 6, 7, 10 and 12. As part of the Danish surveillance program for App, the Danish Veterinary Institute uses ELISAs and complement fixation tests (CFT) to test for porcine anti-App antibodies (2-7). In an effort to improve our diagnostic tools, we are currently developing a novel indirect fluorescent microsphere immunoassay that can facilitate simultaneous detection of antibodies towards multiple App serovars within a single serum sample volume. The multiplex immunoassay is based on Luminex technology (8) and has several benefits compared to ELISA and CFT, including reduced serum sample volumes, lowered amount of labor and faster acquisition of results.

MATERIALS AND METHODS:

The multiplexed assay employs up to 80 batches of microscopic magnetic beads that differ in fluorescence. In our assay, seven batches of beads were successfully coupled with capsular App antigens that were previously purified for use in ELISA and CFT. These antigens included lipopolysaccharide (LPS) extracted from cultures of six App serovars (App 1, 2, 6, 7, 10 and 12) or a mixture of LPS and capsular polysaccharide extracted from App 5. The coupled beads were plexed and incubated with sera from experimentally infected pigs as well as from naturally infected and non-infected pigs. Bound antibody was detected with layers of biotinylated anti-pig antibodies and fluorescently labelled streptavidin. Samples were analyzed in a Bioplex 200 reader (9) and results were read as median fluorescence intensity (MFI).

RESULTS:

The specificity and sensitivity of the multiplex immunoassay were similar to that of our ELISAs. Since we have not previously succeeded in establishing an App 1 ELISA, we currently use CFT to detect infection with this serovar. However, App 1 LPS was successfully coupled to beads and included in the multiplex immunoassay. Antigen-specific reactivities measured in a monoplex format were attained when combining beads coupled with App 1, 2, 5, 6, 7, 10 and 12 in a multiplex format, indicating limited cross-reactivity. In addition, antigen-coupled beads maintained stable interaction with serum antibodies when analyzed over a period of four months. Longer storage periods have not yet been tested.

DISCUSSION AND CONCLUSIONS:

The multiplex assay as designed is a sensitive and specific method for detection of porcine antibodies to App, and shows good overall agreement with our well-established ELISAs. The multiplex assay is rapid and simple and has the potential of being an important addition to current immunoassays for detection of infection with App as well as additional infectious agents in pigs or other production animals.

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